Effect of genetic polymorphism of *αS1- casein* gene on qualitative and quantitative milk traits in native Bulgarian Rhodopean cattle breed

J Peter Hristov[1*], Denitsa Teofanova[3], Ani Georgieva[2], Georgi Radoslavov[1]

[1]Institute of Biodiversity and Ecosystem Research – Bulgarian Academy of Sciences, 25 Acad. Georgy Bonchev Str., 1113 Sofia, Bulgaria

[2]Department of Pathology, Institute of Experimental Morphology, Pathology and Morphology and Anthropology with Museum, Bulgarian Academy of Sciences, 25 Acad. Georgy Bonchev" Str., 1113, Sofia, Bulgaria

[3]Department of Biochemistry, Sofia University "St. Kliment Ohridski", 8 "Dragan Tsankov" blvd. 1164, Sofia, Bulgaria

Corresponding Author: Peter Hristov

E-mail: peter_hristoff@abv.bg

Genet. Mol. Res. 17 (1): gmr16039868

Received December 14, 2017

Accepted January 18, 2018

Published January 28, 2018

DOI http://dx.doi.org/10.4238/gmr16039868

ABSTRACT. Milk protein genetic polymorphisms of the genus Bos provoke a significant scientific interest, mainly associated with their evolution, population structure, breeding and hybridization. The aim of present study is to investigate the influence of the genetic variants of *αS1- casein* gene with respect to milk production and quality of native for Bulgaria cattle breed - Bulgarian Rhodopean cattle. A total of 87 unrelated animals of that breed were examined for genetic variants of the *αS1- casein* gene through PCR-RFLP analysis. The results showed that three genotypes BB, CC and BC were presented with 26.4%, 2.3% and 71.3%, respectively. The frequency of B allele (62.1%) was almost twice higher as compare with C allele of the gene (37.9%). The effect of estimated genotypes on qualitative and quantitative milk traits could be summarized as follow: milk production and milk butter, BC>BB>CC; fat and protein contents, CC>BB>BC. The presence of correlation between genetic variants of *αS1- casein* gene and qualitative and quantitative bovine milk traits gives a reliable approach for selection of animals with desirable milk traits and genetic elaboration of that native breed as a part of national genetic fund.

Key words: Genetic polymorphism; αS1 - casein; Bulgarian Rhodopean cattle; Milk proteins

INTRODUCTION

Over the last decades, numerous studies have been concentrated on the influence of the genetic variants of the major milk proteins on the quantitative and qualitative milk traits and their technological properties (Di Stasio & Mariani, 2000; Martin et al., 2002). Cow milk contains two classes of specific proteins, i.e., the group of caseins and the group of whey proteins. The first class contains four caseins, i.e., αs1- (*CSN1S1*), αs2- (*CSN1S2*), β-(CSN2) and κ-casein (CSN3). Each of the abovementioned proteins is presented by at least two genetic variants. They differ from each other by one or more amino acid residues in the polypeptide chains, which is due to various types of mutations in the genes encoding them. There are several methods for genotyping milk protein polymorphisms; however, the most frequent, cheap, and fast technique is the PCR-RFLP assay. The alleles of a gene can be identified through their restriction profile. Most of the studies have been focused on *CSN1S1* and CSN3 of the group of caseins. These proteins have a great effect on milk production and milk constituents (Erhardt, 1996). *αS1- casein* gene is localized in the Chromosome 6 (Popescu et al., 1996). The genomic DNA encoding the *CSN1S1* milk protein is about 17.5 kb. A recent review of the milk protein nomenclature (Caroli et al., 2009) indicates nine genetic variants of the *αS1- casein* gene in the genus *Bos*. For this gene, the most common allele is B followed by C. These allele forms can be found in all cattle breeds. One of the most important effects of the milk protein polymorphisms on milk traits of economic importance is their relation to the technological properties of milk. Some variants of these genes have a significant influence on the production of cheese, yellow cheese, human nutrition (hypoallergenic milk) etc.

The Bulgarian Rhodopean cattle (BRC) is an ancient local breed that has served as the main livelihood of the human population in the Rhodopa mountains. The breed is characterized by a long period of economic use, unpretentiousness in different climatic conditions as well as breeding and feeding. It has high relative milk ness, resistance to respiratory, contagious and parasitic diseases. The milk production, butter milk and milk protein per 100 kg. Live weight, viability, duration of use and fertility that Bulgarian Rhodope bovine has is unique.

Until now, the selection and breeding of Bulgarian Rhodopean cattle is the main occupation of people in the Rhodopa Mountain. Moreover, the Government of the Republic of Bulgaria encourages the people in this mountainous region of the country to breed those cattle. The people are also stimulated not to admit crossbreeding with introducing more productive cattle breeds. Thus, the Bulgarian Rhodopean cattle is preserved from genetic ingression, which is important to protect pure breed animals.

This is the first comprehensive research that concerns the effect of the genetic variants of *αS1- casein* gene on milk traits in Bulgarian Rhodopean cattle breed.

MATERIAL AND METHODS

Animals and sample collection

A total of 87 blood samples (5 ml) from *v. jugularis* into K_2EDTA were obtained from pure breed unrelated animals of Bulgarian Rhodopean cattle situated in dairy cattle Experimental Station (ASES - Smolyan). The cows were between 3 and 8 years of age and were fed with balanced diet in terms of energy and protein. To investigate milk traits, milk samples were collected from each cow monthly for 300 days of lactation. Milk production, butter milk, fat and protein content were measured by Milk Oscan 133-B (Foss Electric).

DNA extraction and PCR amplification

Total DNA was extracted from blood samples by using of Gene Jet™ Genomic DNA Purification Kit (Thermo Fisher Scientific Inc., Cat. number: K0721, USA) according manufacturer's instruction. The extracted DNA was resuspended in 50 µL of elution buffer. The DNA concentration was determined spectrophotometrically, and the quality of the DNA samples was examined on 1% agarose gel electrophoresis stained with Green safe premium (Cat. No. MB13201, Nzytech, Portugal) under UV light. The purified DNA was stored at –20 °C until PCR assay.

For the amplification of polymorphic segment of *CSN1S1* gene the following primers were used: ALFAS1F 5'-TGCATGTTCTCATAATAACC-3' and ALFAS1R 5'-GAAGAAGCAGCAAGCTGG-3' (Koczan *et al.*, 1993). They covered parts of the 5'-flanking region and exon 1 (in total 310 bp fragment). In addition, a negative control was included for all PCR reactions. The PCR mixture (50 µl total volume) consisted of 10 ng DNA, 0.5 µM of each primer and NZYTaq 2x Colorless Master Mix (Cat. No. MB04002, NZYTECH, Lisbon, Portugal). The amplification conditions were as follows: initial denaturation 95°C for 5 min.; 35 cycles (denaturation 95°C for 30 sec.; primer anealing 50°C for 30 sec.; extension 72°C for 1 min.). The reaction was concluded with a final extension for 10 min at 72°C after the final amplification cycle. PCR products were visualized on 1% agarose gel stained with Greensafe premium (Cat. No. MB13201, Nzytech, Portugal) under UV light. Fragment size was determined using Gene Ruler 1 kb Plus DNA Ladder (Thermo Fisher Scientific Inc., USA).

RFLP assay

All positive reactions (successfully amplified fragments) were restricted with *NmuCI* (*Tsp45I*) specific endonuclease (Cat. number: ER1511, Thermo Fisher Scientific Inc., USA) for 1 hour at 65°C according to manufacturer's instructions. Restriction products were visualized on 2% agarose gel stained with Greensafe premium (Cat. No. MB13201, Nzytech, Portugal) under UV light. Fragment size was determined using GeneRuler 1 kb Plus DNA Ladder (Thermo Fisher Scientific Inc., USA). According to restriction profile allelic variants of *CSN1S1* gene were determined.

Statistical analysis

Milk productivity and qualitative traits data was analyzed by Statistical Tool Descriptive statistics (Microsoft Excel, 2007). Calculated mean values (shown as mean value ± SEM) for milk productivity and qualitative traits were compared within different genotypes. Genotype and allele frequencies were determined. Validity of Hardy-Weinberg equilibrium for the population was evaluated using $\chi 2$ test (Preacher, 2001)

RESULTS AND DISCUSSION

PCR-RFLP assay of *CSN1S1* gene

A total of 87 animals of the BRC breed were examined for genetic variants of the *CSN1S1* gene. Three genotypes were obtained, two homozygous (BB and CC) and one heterozygous (BC) (Table 1). About 71% of the animals (62 cows) were heterozygous and their RFLP profiles showed three electrophoretic bands (310bp, 214 bp and 96 bp). Only 26% (23 cows) were homozygous BB animals and two electrophoretic bands were characteristic for them (214 bp and 96 bp). The homozygous CC genotype was presented by the lowest frequency (2%), which could be pointed out as an insignificant presence. There were only two cows found with that genotype expressed, with one unrestricted fragment on the electropherogram (310 bp).

Genotype and allele frequencies

Distribution of genotype and allele frequencies among the studied animals is presented in Table 1. Genotype frequencies were estimated after a direct count. On the other hand, allelic frequencies were calculated from the observed genotype frequencies.

Table 1. Genotypic and allelic frequencies in the Bulgarian Rhodope cattle population with respect to the α1-casein

Gene	Genotype	Genotypic frequencies		Allelic frequencies	χ^2	p-value
		Observed	Expected			
CSN1S1	BB	0.264	0.385	B – 0.621	0.26 NS	0.88
	CC	0.023	0.144	C - 0.379		
	BC	0.713	0.471			

For the *CSN1S1* gene in BRC breed, it is obvious that the B allele frequency is predominant in comparison with the C allele. This finding agrees with previous studies, which have defined the B allele as being the most frequent in many cattle breeds (Beja-Pereira et al., 2003; Micinski and Klupczynski, 2006; Hristov et al., 2013).

The observed and the expected genotype frequencies were of similar values, thus confirming the validity of Hardy-Weinberg equilibrium for the BRC population. The prevailing frequency of the B allele and the heterozygous BC genotype for the *CSN1S1* gene allowed the assumption that animals possessing the BB, and/or the BC genotypes have been used during the selection and reproduction of the BRC breed. The extremely low frequency of the homozygous CC individuals corroborated with the above-mentioned assumption.

Effect of genetic variants of *CSN1S1* gene on qualitative and quantitative milk traits

With respect to the importance of the *CSN1S1* gene polymorphism for the milk production, it was found that the heterozygous BC animals showed the highest values (3877.32 ± 114.67 kg) (Fig. 1). This exceeded with c. 12% the milk yield of the CC homozygous animals (3412.00 kg ± 103.09 kg) and with 7% that of the BB homozygous cows (3600.81 ± 153.79 kg). Similar results were obtained for butter milk data, where the BC animals had better values and the lowest values were those of the CC cows (BC - 179.93 ± 5.12 kg; BB - 170.06 ± 8.00 kg; CC - 167.01 ± 7.35 kg). These observations allowed the assumption for the superiority of the B allele of the *CSN1S1* gene relative to both above-mentioned milk features. The milk fat and protein contents were affected mainly by the CC genotype. The values of the protein content (CC - 3.72 ± 0.04%; BB - 3.68 ± 0.06%; BC - 3.63 ± 0.03%) were similar and only a slight superiority of the CC genotype was detected. The differences were more obvious with respect to the fat content (CC - 4.88 ± 0.05%; B -, 4.72 ± 0.08%; BC - 4.66 ± 0.04%). With respect to the fat and protein contents, there was predominance of the C allele of the *CSN1S1* gene. The results about qualitative and quantitative milk traits were summarized as follow: milk production and milk butter, BC>BB>CC; fat and protein contents, CC>BB>BC (Figure 1). B allele of the gene is associated with higher milk production, protein content and butter milk as compare with homozygous C animals. On the other hand, C allele seems to be associated with more fat content (Figure 1).

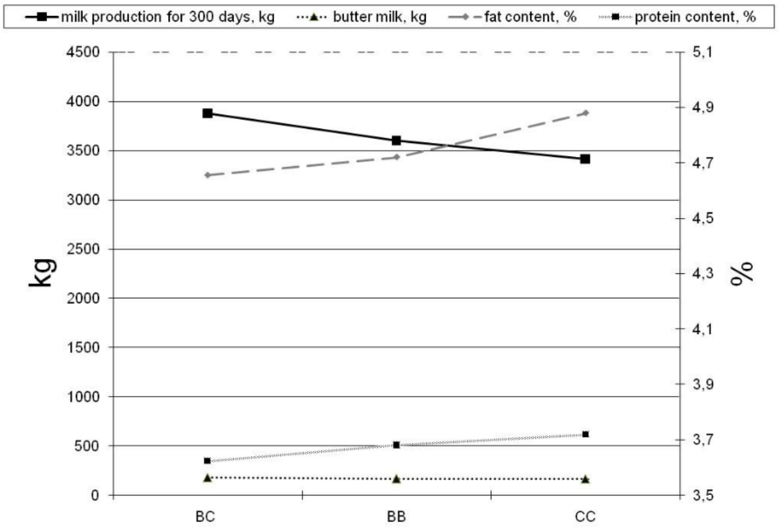

Figure 1. Influence of the *CSN1S1* gene polymorphism on the milk production and the milk quality traits in cows of Bulgarian Rhodopean cattle. BB, CC, BC – genotypes.

The correlations between the *CSN1S1* gene polymorphism and the milk traits obtained by other researchers have not been straightforward, partly due to the differences in parameters used and/or depending on cattle breeds. e.g., the *CSN1S1* BB genotype correlated with higher milk production in some cases (Ng-Kwai-Hang et al., 1984; Aleandri et al., 1990; Sang et al., 1994) but there was also an evidence for the superiority of the heterozygous BC genotype (Micinski et al., 2007). Our results support the positive effect of the BC genotype on the milk yield being about 9.5% higher than the homozygous genotypes. In general, the results presented, and the published data confirm the dominance of the B allele over the C allele relative to the milk production. No publications were found about the influence of the genotypes of the *CSN1S1* gene on the butter milk values; however, the present study revealed a positive effect of the B allele of this gene.

The data concerning the protein content are controversial. According to some reports, the BB genotype is linked to high protein content (Ng-Kwai-Hang et al., 1984; Aleandri et al., 1990; Sang et al., 1994) but the same genotype was associated with low protein values in other studies (Ng-Kwai-Hang et al., 1986, 1992). Micinski et al. (2007) reported that the *CSN1S1* BC genotype affected the increase of the protein content of milk. Our observations coincided with data presented by Pečiulaitienė et al. (2007) that had demonstrated the superiority of the CC genotype relative to the protein content.

Regarding the fat content, all publications claim that the homozygous BB genotype is associated with higher values (Micinski et al., 2007; Kamiński, 1996; Pečiulaitienė et al., 2007). This contrasted with the present results exhibiting c. 4% of higher fat content in the milk of the CC animals compared to the BB cows from BRC breed.

CONCLUSIONS

1. The predominant frequency of the B1 allele of the α1-casein gene was found in the population of the Bulgarian Rhodopes cattle.

2. The heterozygous BC genotype is associated with higher milk production and milk yield.

3. Fat and protein content of cow's milk are higher in the homozygous CC genotype.

4. The correlation between allelic forms of the α1-casein gene and qualitative and quantitative milk traits can be used in the selection and breeding of the Bulgarian Rhodopean cattle for the genetic improvement of the breed.

ACKNOWLEDGMENT

This work was supported by the National Scientific Fund of the Bulgarian Ministry of Education and Science, [grant numbers 06/10 17.12.20016].

REFERENCES

Aleandri RLG, Buttazzoni JC, Schneider A, Caroli RD (1990). The effects of milk protein polymorphisms on milk components and cheese-producing ability. *Journal of dairy science*. 73: 241-255. https://doi.org/10.3168/jds.s0022-0302(90)78667-5

Caroli M, Chessa S, Erhardt GJ (2009). Milk protein polymorphisms in cattle: effect on animal breeding and human nutrition. *Journal of Dairy Science.* 92:5335-5352. https://doi.org/10.3168/jds.2009-2461

Di Stasio LP, Mariani (2000). The role of protein polymorphism in the genetic improvement of milk production. *Zootecnica e Nutrizione* Animale. 26: 69-90.

Erhardt G (1996). Detection of a new κ -casein variant in the milk of pinzgauer cattle. *Animal Genetics*. 27, 105-107. https://doi.org/10.1111/j.1365-2052.1996.tb00477.x

Neov BD, Teofanova L, Zagorchev G, Radoslavov PH (2013). Milk protein polymorphism in Bulgarian grey cattle population. *Bulg. J. Agric. Sci.,* Supplement 2, 19: 194-196.

Kamiński S (1996). Bovine kappa-casein (CASK) gene – molecular nature and application in dairy cattle breeding. *Journal of Applied Genetics.* 37: 179-196.

Koczan DG, Hobom HM, Seyfert P (1993). Characterization of the bovine αS1-casein gene C allele based on a MaeIII polymorphism. *Animal Genetics.* 74: 74. https://doi.org/10.1111/j.1365-2052.1993.tb00935.x

Martin PM. Szymanowska L, Zwierzchowski C, Leroux S (2002). The impact of genetic polymorphisms on the protein composition of ruminants milks. *Reproduction Nutrition Development*. 42: 433-459. https://doi.org/10.1051/rnd:2002036

Miciñski J, Klupczyñski J (2006). Correlations between polymorphic variants of milk proteins, and milk yield and chemical composition in Black-and-White and Jersey cows. *Polish Journal of Food and Nutrition Sciences*. 15(56): 137-143.

Miciński JJ, Klupczyński W, Mordas R, Zablotna (2007). Yield and composition of milk from Jersey cows as dependent on the genetic variants of milk proteins. *Polish Journal of Food and Nutrition Sciences*. 57: 95-99. https://doi.org/10.5194/aab-51-216-2008

Ng-Kwai-Hang KF, Hayes JF, Moxley JE, Monardes HG (1984) Association of genetic variants of casein and milk serum proteins milk, fat, and protein production in dairy cattle. *Journal of Dairy Science*. 67: 835-840. https://doi.org/10.3168/jds.s0022-0302(84)81374-0

Ng-Kwai-Hang, KF, Grosclaude F (1992). Genetic polymorphism of milk proteins. In: Advanced Dairy Chemistry. Fox, P.F. (Ed.), Proteins. 1: 405-455. https://doi.org/10.1007/978-1-4419-8602-3_22

Pečiulaitienė N, Miceikienė I, Mišeikienė R , Krasnopiorova N, et al. (2007) Genetic factors influencing milk production traits in Lithuanian dairy cattle breeds. *Žemės Ūkio Mokslai*. 14: 32-38.

Popescu CP, Long S, Riggs P, Womack J (1996). Standardization of cattle karyotype nomenclature: Report of the committee for the standardization of the cattle karyotype. *Cytogenetics and Cell Genetics*. 74, 259-261. https://doi.org/10.1159/000134429

Preacher KJ, (2001). Calculation for the chi-square test: An interactive calculation tool for chi-square tests of goodness of fit and independence [Computer software].

Sang BC, Ahn BS, Sang BD, Cho YY (1994). Association of genetic variants of milk proteins with lactation traits in Holstein cows. Proceeding of 7th AAAP Animal Science Congress, 2, 211-217. https://doi.org/10.1111/j.1439-0388.1997.tb00529.x